

# Laboratory diagnosis of porcine reproductive and respiratory syndrome (PRRS) virus infection

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**Summary:** *Porcine reproductive and respiratory syndrome (PRRS) is a relatively new, economically important disease of pigs and is characterized by reproductive failure in sows and gilts, pneumonia in young growing pigs, and an increase in preweaning mortality. The PRRS virus (PRRSV) was initially isolated in the Netherlands and the United States and was labeled as the Lelystad and ATCC VR-2332 viruses, respectively. Although these two strains are antigenically similar, some genetic and antigenic differences have been reported. Laboratory diagnosis of PRRSV infection can be made on the basis of virus isolation or viral serology. Recently, a polymerase chain reaction (PCR) test has been developed to detect PRRSV in boar semen and swine sera. The purpose of this diagnostic note is to provide a brief update on the laboratory methods used to diagnose PRRSV infection in swine.*

## PRRS serological testing is subject to the same guidelines as serological tests for other swine diseases

Acute and convalescent (paired) serum samples should be provided to a diagnostic laboratory when seeking evidence of recent infection.

The vaccination status and potential presence of passive antibodies should be considered when evaluating serologic results.

- A recent European publication indicates that passively acquired PRRS virus (PRRSV) antibodies, as measured by blocking ELISA, can be detected in pigs until 4–10 weeks of age.
- Serological tests that distinguish vaccinated from naturally infected swine are not available at the present time.

Appropriate sample size depends on the expected prevalence of infection, the size of the population, and the level of confidence desired.

In general:

- A sample size of 30 provides a 95% degree of confidence for detecting a prevalence of at least 10%.
- A sample size of 10 provides a 95% degree of confidence for detecting a prevalence of at least 30%.

## Diagnostic notes are not peer-reviewed.

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- In single-site, farrow-to-finish swine herds, the seroprevalence of PRRSV infection is usually highest in the grow-finish unit. Serum from 10 finishing pigs is usually an adequate sample size to determine whether the herd has been infected with PRRSV. In multisite production systems, each stage of production represents a single population, so each site should be sampled.

A negative PRRS serologic result at one sampling time has several possible interpretations:

- the pig was not infected with PRRSV;
- the pig was recently infected with PRRSV and has not yet seroconverted;
- the pig was infected with PRRSV but has become seronegative; or
- the test employed for PRRS antibody detection was negative because of low test sensitivity or laboratory error.

## Serological diagnosis of PRRSV infection

Various serological tests have been developed to detect anti-PRRSV antibody in swine sera. The following summary on PRRSV serological tests is restricted to tests that are most widely available in the United States and Canada, namely indirect-fluorescent antibody (IFA), enzyme-linked immunosorbent assay (ELISA), and serum neutralization (SN) tests.

### Indirect fluorescent antibody test (IFA)

IFA antibodies (IgG) appear by 7–11 days post infection, peak at 30–50 days post infection, and may decline to undetectable levels by 4–6 months (Table 1).

The IFA has a high specificity (99.5%) but unknown sensitivity for individual animals.

An advantage of the IFA test compared to ELISA is that the magnitude of the titer can be determined.

- Titers are usually reported as fourfold dilutions ranging from 16 to 1024, although some laboratories begin the initial dilution at 20.
- A titer of 16 or 20 is considered positive.
- IFA titer endpoints are subjectively determined, resulting in test result variation among technicians and laboratories performing the test.
- The IFA test will only detect antibodies to strains of PRRSV closely related to the PRRSV strain employed in the test. Two different IFA

Table 1

Serological tests for porcine reproductive and respiratory syndrome (PRRS) virus

Serological test	Antibody first detected	Peak antibody titer	Decline in antibody titer	Antibody undetectable at	Positive titer	Sensitivity	Specificity
Indirect Fluorescent Antibody (IFA) (Detects IgG)	7–11 days PI <sup>a</sup>	30–50 days PI	Rapid	4–6 months PI	≥1:16 or ≥1:20 <sup>b</sup>	?	High
Indirect Fluorescent Antibody (IFA) (Detects IgM)	5 days PI in 3-week-old pigs; 7 days PI in sows	14 days PI	Very rapid	28 days PI in 3-week-old pigs; 21 days PI in sows	≥1:16 or ≥1:20 <sup>b</sup>	?	?
ELISA	9–13 days PI	30–50 days PI	Rapid	4–≥10 months PI	S:P ratio ≥0.4	High <sup>c</sup>	High
Serum Neutralization	9–28 days PI	60–90 days PI	Gradual	≥ 1 year PI	≥1:4	Low	High

<sup>a</sup> PI = post infection

<sup>b</sup> Depends on the initial dilution used in the IFA test

<sup>c</sup> Sensitivity (100%, 35/35 samples) and specificity (99.5%, 413/415 samples); personal communication, Michael L. Snyder, IDEXX Laboratories Inc.

tests, one using a United States strain of PRRSV and another using a European strain (Lelystad strain), are required to detect antigenically diverse PRRSV isolates. Thus, technician labor and supply costs are increased.

- The IFA test is not automated, so it is difficult to perform on a large scale. For the aforementioned reasons, many diagnostic laboratories are using automated PRRS ELISA for routine testing.

An IFA test to detect IgM antibodies against PRRSV has been developed and used experimentally.

- The hypothesis is that detecting IgM antibodies provides evidence of recent infection.
- Problems with nonspecificity of the IgM IFA test must be corrected before this test is used for diagnostic purposes.
- Serum for IgM testing should be refrigerated... **not** frozen.

### PRRS ELISA (HerdChek-PRRS®; IDEXX Laboratories Inc.; Westbrook, Maine)

This test is reported to have high sensitivity and specificity:

- sensitivity of 100% (35 of 35 samples) and specificity of 99.5% (413 of 415 samples).

ELISA antibodies appear by 9–13 days post infection, rise to peak values by 30–50 days post infection, and then decline (Table 1). Estimates are that ELISA antibodies exist at detectable levels for approximately 4–≥10 months. Note that the kinetics of PRRS antibody formation and decay as detected by IFA and ELISA are similar.

PRRS ELISA offers several advantages:

- automation;
- less variation because a machine, rather than a technician, interprets the results;

- detects both United States and European PRRSV strains;
- rapid results; and
- the test is licensed by the USDA and AgCanada.

The presence of PRRSV antibodies is determined by measuring the sample to positive ratio (S:P ratio) which is corrected for nonspecificity.

- A ratio of 0.4 or greater is considered positive.
- Although the magnitude of the S:P ratio might be correlated with the magnitude of IFA titer, the manufacturer does not recommend interpreting the S:P ratio in this way at this time. Research on a PRRS herd profiling quantitative method is under development.

### PRRS serum neutralization (SN)

The serum neutralization test is less sensitive than IFA or ELISA.

- Antibodies are slow to appear; they are first detected 9–28 days post infection.
- SN titers rise slowly for approximately 2–3 months and then gradually decline.
- Maximal SN titers range from 64–≥256.
- SN antibodies persist longer than IFA or ELISA.
- PRRS SN antibodies have recently been estimated to exist at detectable levels for at least 1 year.

PRRS SN testing is not widely used at the present time.

### Detecting PRRSV in tissues

#### Histopathology

Interstitial pneumonia is the most characteristic histologic lesion of PRRSV infection. Pulmonary lesions are characterized by three main changes:

- alveolar walls thickened by macrophages and lymphocytes;
- type II pneumocyte hypertrophy and hyperplasia; and
- accumulations of necrotic debris and mixed inflammatory cells in alveolar spaces.

Other PRRSV lesions observed less frequently include lymphohistiocytic myocarditis, rhinitis, and encephalitis.

Lung, heart, brain, tonsil, and nasal turbinate fixed in 10% neutral buffered formalin should be submitted for histologic examination.

### Direct fluorescent antibody test (DFA)

The DFA test is useful for detecting PRRS viral antigen in tissues. The DFA test is rapid and economical; fresh tissue (preferably lung) is frozen at  $-70^{\circ}\text{C}$ , sectioned using a cryostat, and stained with an anti-PRRSV monoclonal antibody fluorescein conjugate. The test is then read using a fluorescent microscope. Total test time is 2–4 hours.

The DFA test is specific but it is not always very sensitive, especially if tissue is autolyzed.

### Immunohistochemistry

- This test may be used to detect PRRS viral antigen in formalin-fixed tissues. It is a more time consuming (24 hours) and a more expensive test to perform than DFA.
- In the research laboratory, immunohistochemistry is more sensitive than DFA.
- Tonsil and lung fixed in **10% neutral buffered formalin** are the preferred specimens for immunohistochemical detection of PRRSV. The type of fixative used, the length of fixation, and the immunohistochemical method employed influence the quality of the results.

### Virus isolation

Serum is the preferred specimen for virus isolation because pigs are viremic for a prolonged time (1–6 weeks or longer). Also, PRRS viruses are more stable in serum than in tissues.

PRRSV can be isolated from many tissues including lung, tonsil, and lymph nodes; however, tissue must be fresh if virus isolation is to be successful. PRRSV is easily degraded by heat and autolysis.

- The virus retains infectivity for 1 month when refrigerated at  $4^{\circ}\text{C}$  and for at least 18 months when frozen at  $-70^{\circ}\text{C}$ ; however, it is rapidly inactivated at  $37^{\circ}\text{C}$ .
- Refrigeration during transport to a diagnostic laboratory helps preserve the virus.

Because autolysis degrades the virus, PRRS viruses are seldom isolated from fetuses.

- Serum from littermates of weakborn or stillborn piglets and from affected sows should accompany fetuses to the laboratory in cases of reproductive failure.

Serum from vaccinated pigs should not be used for virus isolation because PRRS-vaccinated pigs are viremic for 3–6 weeks. Vaccine strain of PRRSV cannot be distinguished from PRRS field strains by routinely available diagnostic tests.

## Detecting PRRSV in semen by PCR

### Polymerase chain reaction (PCR)

PCR is a highly sensitive test that can detect as few as 10 TCID<sub>50</sub> of PRRSV.

The primary diagnostic application of PCR has been detecting PRRS viral RNA in semen of boars.

In most cases, a positive PCR on semen also results in a positive swine bioassay, indicating that the RNA detected in semen represents infectious virus.

The PCR test is available at the South Dakota Veterinary Diagnostic Laboratory, Box 2175, Brookings, SD, 57007, USA, phone (605) 688-5171; or Manitoba Veterinary Services Laboratory, 545 University Crescent, Winnipeg, Manitoba, Canada, R3T 5SG, phone (204) 945-8838.

- Semen sample(s) should be frozen.
- At least three successive semen samples at weekly intervals should be tested because PRRSV may be shed intermittently. Semen samples (3–5) may be pooled for PCR testing.

*Editor's note: Please see accompanying article on PRRS virus detection in semen by Jane Christopher-Hennings, et al., appearing next in this issue.*

### References

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